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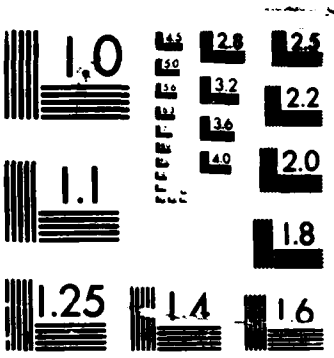

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REPORT 5

A PROGRAM FOR STUDY OF SKELETAL MUSCLE CATABOLISM FOLLOWING PHYSICAL TRAUMA

Annual/Final Report
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Douglas W. Wilmore, M.D.
Department of Surgery
Harvard Medical School and the
Brigham and Women's Hospital
75 Francis Street
Boston, Massachusetts 02115

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This contract examines methods of attenuating post-traumatic metabolic responses. The increased glucose production which occurs following infection and injury in sheep was not decreased by infusion of β-hydroxybutyrate; similar studies in normal or fasting animals demonstrated prompt attenuation. Net proteolysis following operation was reduced by amino acid infusion. Solutions fortified with branched chain amino acids exerted no beneficial effect. Post traumatic skeletal muscle proteolysis could be attenuated by α and β adreneric blockade or epidural anesthesia. Skeletal muscle nitrogen balance could be predicted from branched chain amino acid uptake and changes of amino acid concentrations in the free amino acid pool.

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FOREWARD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1987).

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I. SUMMARY OF PREVIOUS REPORTS

1. "Annual Summary Report", July 1980 - July 1981
"Annual Summary Report", August 1981 - September 1982

To determine if the ketonemia following injury contributed to the increased glucogenesis associated with this catabolic disorder, glucose production and arterial substrates were measured before and after infusion of Na-DL-ß-hydroxybutyrate (ß-OHB, 20 Mol/kg · minute) in fed, fasted, and fasted-infected sheep. Following three hours of ß-OHB infusion in the awake, conditioned animals, ß-OHB and acetoacetate blood concentrations more than doubled. With infusion, blood glucose and alanine concentrations decreased in the fed and fasted sheep but not in the fasted-infected group. Glucose production fell significantly from 10.11±1.33 µMol/kg · min to 8.44±1.05 in the fed animals, and from 5.05±0.28 to 4.11±0.33 in the fasted group. Glucose production was unaffected by ß-OHB infusion in the fasted-infected animals (9.50±1.83 versus 9.11±1.44). The accelerated rate of glucose production in sheep following infection is not a consequence of the hypoketonemic state associated with sepsis.

To determine the effect of the endorphine system on post-traumatic/septic metabolic responses, Naloxone (2 mg, I.V.) was administered to four sheep, before and after infection. In these normotensive animals, no major alteration in substrate concentration was noted. The endorphine system does not appear to exert major metabolic regulatory effects in this model.

2. "Annual Summary Report", September 1982 - August 1984
"Annual Summary Report", September 1984 - November 1985

The purpose of this work was to attenuate skeletal muscle proteolysis in the post-traumatic period. In the initial study, amino acid solutions were administered with or without glutamine supplementation. Amino acid administration at the dose of 0.624 grams N/kg · hour was associated with near nitrogen balance, maintenance of skeletal muscle intracelluar stores, and attenuation of hindquarter nitrogen loss. Glutamine enriched solutions were as effective as standard balanced formulas in sparing body protein.

In a second study, amino acid formulas were constructed to provide a range of concentration of branched chain amino acids (from 12-44%). Iso-nitrogenous infusions were administered to animals following operations and the effects of the various formulas determined. No benefit was derived from branched chain enriched solutions over a standard formula.

In additional studies, adrenergic blockade was achieved by administering phentolamine and propranolol or utilizing high epidural anesthesia. While blockade did not reduce nitrogen excretion in the post-traumatic period, nitrogen efflux from the hindquarter was markedly attenuated. This is the first demonstration of a relationship between the adrenergic nervous system and accelerated proteolysis. The significance of these findings is discussed.

II. SUMMARY OF CURRENT REPORT

Amino acid solutions rich in branched chain amino acids (BCAA) are commonly utilized both clinically and in experimental protocols in an attempt to reduce skeletal muscle and whole body protein catabolism. To investigate the effectiveness of BCAA infusion, amino acid formulas containing varying concentrations of BCAA were given perioperatively in this study to three groups of dogs undergoing a standard laparotomy and retroperitoneal dissection. A fourth group was given saline alone. Using previously described hindquarter flux techniques, individual and total amino acid nitrogen exchange rates were measured and utilized in estimating skeletal muscle protein catabolism. Intracellular free amino acid concentrations were measured in percutaneous muscle biopsy samples. Although there was no relationship with the rate of BCAA infusion, there was a significant correlation between the rate of BCAA uptake by muscle and diminished total nitrogen release from hindquarter skeletal muscle postoperatively. There was also a significant relationship between muscle nitrogen balance and the post-operative change in the muscle concentration of either total amino acids or the single amino acid glutamine. When combined in a single equation BCAA uptake and the change in muscle free amino acid concentration predict skeletal muscle nitrogen release with an $R = .86$. Thus the rate of BCAA uptake and the free glutamine or total amino acid concentration in muscle appear to be independent predictors of muscle nitrogen balance. The nitrogen-sparing effect of BCAA in skeletal muscle is unrelated to infusion concentration or rate of infusion

III. BRANCHED CHAIN AMINO ACID UPTAKE AND MUSCLE FREE AMINO ACID CONCENTRATIONS PREDICT POST-OPERATIVE MUSCLE NITROGEN BALANCE

There is a net breakdown of body protein after major operations, accidental injury, septicemia or other critical illnesses. Much of the protein loss occurs in skeletal muscle as demonstrated by the increased release of amino acids from muscle[1] and the marked wasting of muscle mass following severe illness.[2] The liberated amino acids are utilized by visceral organs such as the gastrointestinal tract liver and kidney as metabolic fuels and as substrates for gluconeogenesis, ammoniagenesis, and acute-phase protein synthesis.[3] These metabolic reactions frequently result in deamination of amino acids with the liberated nitrogen routed into hepatic ureagenic pathways and ultimately excreted from the body as urea. The accelerated release of amino acids from skeletal muscle protein is therefore associated with increased total body nitrogen loss.[2] Because visceral amino acid uptake exceeds skeletal muscle amino acid release, the plasma levels of many amino acids fall.[4] Administration of intravenous amino acids to catabolic patients may often be necessary to maintain normal plasma concentrations, satisfy visceral amino acid requirements, spare skeletal muscle protein, and preserve lean body mass.

III. (Continued)

The branched chain amino acids (BCAA) valine, leucine, and isoleucine are essential amino acids which are thought to have important influence on skeletal muscle protein turnover. These three amino acids serve as precursors for muscle protein synthesis, and also can be oxidized in muscle as a source of metabolic energy.[5] In addition, leucine has been shown to stimulate protein synthesis and to inhibit protein degradation in vitro.[6] When infused into postoperative rats, either singly or in combination, BCAA's were found to have a beneficial effect on overall protein turnover.[7,8] This suggested that a higher than standard concentration of infused BCAA's might be more efficient in overcoming protein catabolism. Other animal work, however, has shown no benefit from high BCAA solutions as compared to standard formulas.[9] The response to infusion of balanced formulas (18-25% BCAA's) or solutions containing high concentrations of BCAA (40-50% of infused amino acids) has also been studied in critically ill humans.[10-12] Some investigators report that the patients receiving the fortified BCAA solutions conserve more nitrogen.[10] They also contend that more skeletal muscle protein is conserved and immunologic function improved following catabolic insults.[10] Other studies have failed to demonstrate these responses.[12]

This work focuses on the effects of intravenous amino acid solutions containing varying concentrations of BCAA's on the regulation of skeletal muscle amino acid metabolism following a standardized surgical procedure in the dog. Previous studies with this model of postoperative catabolism have demonstrated marked increases in skeletal muscle protein breakdown and urinary nitrogen excretion.[13] By measuring hindquarter amino acid flux and free amino acid concentrations in plasma and skeletal muscle during the first 24 hours following operation, it has been possible to evaluate the anticatabolic response to the infusion of BCAA's and other amino acids.

Materials and Methods

Preparation of Animals and Sequence of Study

Twenty-seven male and non-pregnant female mongrel dogs were obtained from a farm where they had been conditioned and screened for parasites. The dogs weighed between 18 and 40 kilograms and were housed for at least one week prior to study in the Harvard Medical School animal care facility. All procedures were in accordance with the guidelines of the Committee on Animals at Harvard Medical School and the Committee on Care and Use of Laboratory Animals of the Institute for Laboratory Animal Resources, the National Research Council (DHEW Publication NIH #78-23, revised, 1978). The animals were kept in individual kennels with 24-hour light exposure and were exercised each morning. Water was provided ad libitum and a single daily feeding of Pro-Pet Respond 2000 dry dog food (Syracuse, New York, at least 25% protein by weight) was provided between 1:00 and 3:00 p.m. The animals were trained to rest quietly in a Pavlov sling prior to study.

III. (Continued)

All food was removed from the kennels at 5:00 p.m. the night before basal studies or operation. Basal studies were performed at 8:00 a.m. after the animal was exercised and placed in the sling.

These studies consisted of the collection of a blood sample from a cannulated foreleg vein for plasma amino acid determination and a percutaneous needle biopsy of the vastus lateralis muscle performed under sodium thiopental anesthesis (Abbott, North Chicago, Illinois, 5 mg/kg body weight, I.V.) to quantitate intracellular free amino acids. After the biopsy, with the dog still anesthetized, a 5 ml sample of arterial blood was obtained by percutaneous puncture of the femoral artery for analysis of whole blood amino acids.

The animal was allowed to recover for three days before further studies were performed. At 7:00 a.m. on the day of operation, again after an overnight fast, the animal was exercised and taken to the operating room where it was anesthetized with sodium pentobarbital (Abbott, North Chicago, Illinois, 30 mg/kg body weight, I.V.) via a foreleg cannula. An endotracheal tube was placed and the animal was allowed to breathe spontaneously a mixture of room air and oxygen provided at 5 L/minute. The dog was placed on an operating table in the supine position and a 16-Fr. catheter was placed percutaneously into the superior vena cava via the external jugular vein. After noting the starting time, an infusion of either saline or the appropriate test amino acid solution was begun via this central catheter with an IMED pump (San Diego, California). Cephalothin (Lilly, Indianapolis, Indiana, 1 gram, I.V.) was given immediately before and upon completion of the operation. The urinary bladder was catheterized and, after discarding residual urine, a closed drainage collectionw as begun at the start of the infusion and carried on for 24 hours. Urine was also collected for a second 24-hur period, with the animal in a metabolic cage after termination of the I.V. infusion.

The abdomen and flanks of the dog were shaved, washed with soap and water, and prepped with a providone iodine solution. The animal was sterilely draped, and the abdomen was entered via an infra-unbilical midline incision in females and a right paramedian incision in males. The bowel was retracted aside, and the retroperitoneum exposed for complete dissection around the distal aorta and inferior vena cave. The right deep circumflex iliac artery and vein as well as the right internal iliac artery were isolated. The two arteries were cannulated with specially prepared catheters consisting of a 6-cm segment of polyethylene tubing (2.08 mm O.D.) linked to 28 mm O.D. polyethylene tubing. One arterial catheter was positioned 6 cm proximally into the aorta via the circumflex iliac artery and the other catheter positioned on cm proximal to the aortic bifurcation, but distal to the caudal mesenteric artery, via the internal iliac artery. A third catheter was inserted into the inferior vana cava via the deep circumflex iliac vein and positioned distal to the renal vein. All catheters were secured and exteriorized through stab wounds

III. (Continued)

in the right flank. The abdomen was closed in layers and the animal turned on its left side. The exteriorized catheters were cut to appropriate lengths, plugged with blunt needles, capped with intermittent injection ports (Jelco, Critikon, Tampa, Florida), flushed with saline, filled with heparin (100 μU/ml), and buried subcutaneously. The injection ports were positioned high in the flanks, allowing easy access to arterial (aortic) and venous (vena caval) blood by percutaneous puncture.

Following these procedures, which generally took two hours, the animal was placed on its side and body temperature was maintained with blankets during recovery from anesthesia. Five hours after the start of the infusion and operation, the animal was placed in the Pavlov sling and a solution of 0.5% para-aminohippurate (PAH) was infused at a rate of 0.7 ml/minute with a Harvard pump into the distal aortic catheter. After 40 minutes of dye infusion, three sets of simultaneous arterial and venous samples were obtained at 10-minute intervals for measurement of amino acid and PAH concentrations. The catheters were then flushed and filled with heparin. The animal was kept in the sling under constant surveillance until the hindquarter flux studies were repeated 24 hours after the start of the infusion. At this point, the first 24-hour urine collection was terminated, and a repeat percutaneous hindlimb biopsy was performed on the leg not previously biopsied, again under brief general anesthesia. The infusion was then terminated and the animal placed in a metabolic cage for the second 24-hour period.

Infusion Solutions

All solutions were infused at the rate of 4 ml/minute/kg. Five control animals received 0.9% saline. Amino acid solutions (Table I) containing BCAA's at three different concentrations (11%, 22%, or 44% of total amino acids) were prepared by adding amino acids to an 8.5% standard amino acid formula, FreAmine III (American McGaw, Irvine, California). The total BCAA infusion rates were 0.46, 0.92, and 1.84 grams/24 hours/kg, respectively. All three amino acid solutions were isonitrogenous, providing approximately 0.624 grams of nitrogen/24 hours/kg, with a constant ratio of valine to leucine to isoleucine (1:1.38:1.05). Nine animals received an 11% BCAA solution which was made by dissolving a mixture of non-essential amino acids (NEAA) in 2.13% FreAmine III to make a solution that provided 0.624 grams of nitrogen/24 hours/kg. In 6 animals, NEAA consisted of L-glutamine alone and in three, NEAA consisted of a mixture of all of the NEAA found in FreAmine III (alanine, glycine, arginine, histidine, serine, and proline) in the same ratios as in FreAmine III. Six animals received 4.25% FreAmine III alone (22% BCAA). The final 7 animals received 2.13% FreAmine III supplemented with enough BCAA's to make a 44% solution. This final formula was made isonitrogenous by adding NEAA as L-glutamine alone (n = 4) or a mixture of the NEAA found in FreAmine III (n = 3). All solutions were sterilized by passage through a 0.22 μM filter (Millipore, Millis, MA) and stored overnight at 4° C prior to administration. A 10-ml sample of each solution was taken at the end of the infusion period and stored at -20°C for analysis of nitrogen by the macro-Kjeldahl method.[14]

III. (Continued)

Preparation and Analysis of Blood, Tissue, and Urine Samples

Whole blood and plasma samples were deproteinized by adding an equal volume of ice cold 10% perchloric acid (PCA) and then centrifuging at 7000 rpm at 4°C for 20 minutes. A 2-ml aliquot of the supernatant was buffered with 0.3 ml of 0.2M sodium acetate buffer (pH = 4.90), adjusted to pH 4.75-4.90 with 5N potassium hydroxide, brought to a final volume of 4 ml with distilled water, and centrifuged again. The resulting supernatant was stored at -20°C for later batch analysis.

During the muscle biopsy procedure, a stop watch was started at the time of tissue removal. The muscle was dissected free of fat and connective tissue and divided into two unequal portions. Multiple weights on each sample were recorded at 15-second intervals for one minute, and the initial muscle wet weight at time = 0 was calculated from the best fit linear regression of weight plotted against time. The smaller sample (approximately 15-20 mg) was dried to a constant weight in an oven at 90°C, and the weight of dried fat-free solids was obtained after extraction in petroleum ether. The sample was then soaked in 250 ml of 1N nitric acid, and the chloride content was measured by titration with silver nitrate using a semi-automated titrator (Radiometer, Copenhagen). Plasma chloride was also determined by a similar method. Intracellular and extracellular water were then calculated using the chloride technique, as previously described.[15] The second muscle sample (approximately 80-100 mg) was weighed and homogenized in 0.5 ml of ice cold PCA using a Polytron homogenizer (Brinkmann, Westbury, New York). The homogenate was centrifuged, and the supernatant was prepared for analysis by addition of buffer and by pH adjustment to pH 4.75-4.90 as described for blood and plasma samples.

Whole blood, plasma, and muscle intracellular glutamine and glutamate concentrations were determined by an enzymatic microfluorometric method modified from the method of Lund,[16] or by automated high performance liquid chromatography (HPLC) after pre-column derivatization with O-phthalaldehyde.[17] The two techniques yielded comparable results. Other amino acids except proline, cystine, and lysine were determined with a similar HPLC method. The concentration of PAH in the arterial and venous blood was determined spectrophotometrically following deproteinization with 5% trichloroacetic acid.[18]

Urine excreted during the 24 hours of infusion was collected in a closed urinary drainage system and stored in acidified, refrigerated containers. Aliquots were stored frozen at -20°C for later batch analysis of nitrogen by the macro-Kjeldahl method.[14] Another portion was centrifuged for 10 minutes at 2000 rpm and frozen for later analysis of urea and creatinine on the Technicon Auto-analyzer (Tarrytown, New York).

III. (Continued)

Calculations and Statistical Analysis

Hindquarter bloodflow was calculated as previously described.[18] Flux rates for the individual amino acids were calculated as the product of bloodflow and arteriovenous concentration difference. Three sets of samples were drawn at each time point, the flux was calculated for each set, and the mean of the three values was determined. Total amino acid nitrogen flux as well as plasma, whole blood, and intracellular nitrogen concentrations were calculated as the millimolar sum of the nitrogen groups of all amino acids measured. Skeletal muscle free intracellular amino acid concentrations were expressed per liter of intracellular water.

Statistical calculations were performed using a standard statistical package (Minitab, The Pennsylvania State University, State College, PA, 1983). The results are expressed as mean ± SEM. Paired and unpaired Student's t-tests were used as appropriate. Analysis of variance was used for multiple group comparisons. Regression analysis was performed using the method of least squares.

Results

All animals survived the operative procedure except for one dog that died shortly after administration of sodium pentobarbital, before the start of the intravenous infusion. This animal was not included in the study. Blood loss during the procedure was uniformly minimal. All sample catheters were patent at the 6- and 24-hour time points, with the exception of one venous catheter at the 24-hour time point in an animal in the 22% BCAA group.

Hindquarter bloodflow at 6 hours was 36.1±6.8 ml/minute/kg in the saline control group and was not affected by treatment (11% BCAA, 33.3±4.9; 22% BCAA, 42.4±8.8; 44% BCAA, 28.7±3.5; differences not significant). Flow at 24 hours was unchanged (57.9±10.2, 38.6±8.2, 54.9±6.5, 49.7±13.2, respectively). The tendency toward higher flow rates and increased variability at 24 hours may be attributable to greater motor activity of the animals following from anesthesia recovery.

Urinary Nitrogen Excretion and Nitrogen Balance

Following operation, the volume of urine excreted was comparable in the four treatment groups, although the dogs receiving saline alone tended to excrete less urine volume (Table II). Urinary nitrogen excretion averaged 0.492±0.020 grams/24 hours/kg in the saline group. The amino acid treated animals excreted 35-65% more nitrogen than the saline group, primarily in the form of urea. The dogs infused with the 22% BCAA solution excreted significantly less urea nitrogen and less total nitrogen than the 11% or 44% BCAA groups. Excretion of creatinine and ammonia was comparable in all groups.

III. (Continued)

Blood urea nitrogen and plasma creatinine were measured before and 24 hours following operation in selected animals from all groups. These concentrations were normal in all animals before operation and fell slightly or did not change postoperatively. Thus, the rate of urinary excretion of urea was similar to the rate of urea production; the higher urea production observed in the animals receiving the 11% and 44% BCAA solutions was significantly related ($p<0.05$) to the extra nitrogen provided by addition of BCAA or NEAA to the balanced amino acid mixture.

Nitrogen balance was less negative with amino acid administration; approximately 50% of the infused amino acid nitrogen was retained. Because nitrogen intake was the same in all animals receiving amino acids, the alterations in nitrogen excretion already discussed were reflected in nitrogen balance (Table II). Thus, the animals receiving the 22% balanced amino acid solution achieved significantly greater nitrogen retention than the dogs receiving solutions containine 11% or 44% BCAA.

Whole Blood Amino Acid Concentrations

In the saline-treated animals, whole blood amino acid nitrogen fell at 6 hours postoperation, but returned to normal preoperative levels by 24 hours (Table III). This transient hypoaminoacidemia was accounted for in large part by a decrease in the concentration of the non-essential amino acids (glutamine, alanine, arginine, serine, and asparagine), although significant decreases in some essential amino acids also occurred (threonine and tyrosine). In contrast, the animals receiving amino acid infusions maintained whole blood amino acid nitrogen concentrations at 6 hours postoperation. These levels increased above preoperative control levels by 24 hours ($p<0.05$).

Concentrations of specific amino acids in the blood of the animals receiving amino acid infusions reflected the composition of the solutions infused. For example, BCAA concentrations wree related to the rate of BCAA administration at both 6 and 24 hours (Figure 1). In general, whole blood glutamine concentrations at 6 hours were lower than preoperative levels (Table III). The exception was the group receiving glutamine enriched 11% BCAA solution, in which the blood glutamine concentration was maintained. In these animals, glutamine comprised more than one-half of the non-essential nitrogen and accounted for more than 40% of the total amino acids delivered. By 24 hours the animals receiving glutamine-containing solutions tended to have higher than normal whole blood glutamine concentrations.

Skeletal Muscle Intracellular Free Amino Acids

In the saline-treated animals, intracellular free amino acid nitrogen fell significantly by 24 hours post-operation when compared to preoperative levels (Table IV). This change was accounted in large part (65%) by the marked fall in intracellular glutamine, which comprised a major portion of the total intracellular free amino acid pool. In the animals receiving amino acid infusions, intracellular nitrogen was maintained, although

III. (Continued)

intraceullar glutamine fell in the animals that received the glutamine-free 11% BCAA solution. Intraceullar glutamine tended to increase in the animals receiving glutamine-enriched solutions and BCAA concentrations increased in proportion to the rate of BCAA infusion.

Hindquarter Amino Acid Flux

In the saline-titrated animals there was net release of amino acid nitrogen from the hindquarter at 6 hours postoperation (Table V). This increased amino acid efflux reflected accelerated release of almost all amino acids measured, including the BCAA., At this time period, glutamate and aspartate were the only amino acids that maintained balance across the hindquarter. At 24 hours postoperation, the rate of hindquarter amino acid nitrogen release had diminished and, although highly variable, the arteriovenous differences for almost all amino acids could not be distinghished from zero. Glutamine efflux persisted at this time point.

In all groups of animals receiving amino acid infusions, hindquarter amino acid nitrogen efflux at 6 hours was similar and was significantly less than in the saline-treated animals ($p<0.05$). Both glutamine and alanine efflux at 6 hours tended to be less in the animals receiving amino acids than in the saline controls. While BCAA's were released at 6 hours in the saline-infused animals, these amino acids were taken up in the dogs receiving amino acid infusions. BCAA hindquarter uptake was related to the rate of BCAA administration (Figure 2) and whole blood BCAA concentrations (Figure 3).

At 24 hours, hindquarter amino acid nitrogen efflux was similar in all the amino acid infusion groups and unchanged compared to 6 hours. At 24 hours, BCAA hindquarter exchange was slightly positive, tending to be greater in the 22% and 44% BCAA groups. At this time, BCAA uptake was unrelated to blood concentrations and rate of BCAA administration.

Relationship Between BCAA Infusion, BCAA Hindquarter Uptake, and Hindquarter Amino Acid Nitrogen Release

In the saline-infused dogs at 6 hours, hindquarter BCAA release was associated with accelerated amino acid efflux. In the animals receiving amino acid infusions, the hindquarter nitrogen balance correlated with BCAA uptake (Figure 4). Saline controls were not yet included in this analysis since they were not receiving nitrogen; inclusion of control animals would have resulted in a regression line with a more positive slope. The correlation was maintained even if BCAA flux was not included in the summation of hindquarter amino acid nitrogen flux ($p<0.02$, $r = 0.49$). Thus nitrogen flux did not correlate with total BCAA concentration in the blood or the rate of BCAA administration. None of these relationships existed at the 24-hour time point.

III (Continued)

Hindquarter amino acid nitrogen release at 6 hours also correlated with changes in the intracellular free amino acid nitrogen pool (Figure 5). Alterations in intracellular glutamine were closely related to changes in the total free amino acid nitrogen pool ($p<0.001$, $r = .90$) and, thus, changes in the glutamine pool were also significantly related to hindquarter nitrogen efflux ($p<0.05$; $r = .66$). These mathematical relationships were maintained when hindquarter amino acid nitrogen efflux was corrected for changes in the intraceullar nitrogen pool. Since the flux and pool measurements were made at different points in time, we made this correction by assuming two different rates of change in the intraceullar amino acid pool. First, we assumed that the change in the intracellular pool occurred in the first 6 hours postoperatively. Alternatively, we assumed that the change occurred at a constant rate over 24 hours. Neither correction altered the relationship between the change in the intracellular nitrogen pool and amino acid nitrogen efflux.

BCAA uptake was not related to increased glutamine release from the hindquarter at 6 or 24 hours. BCAA uptake was also unrelated to the changes that occurred in the skeletal muscle intracellular nitrogen pool and amino acid nitrogen efflux.

BCAA uptake was not related to increased glutamine release from the hindquarter at 6 or 24 hours. BCAA uptake was also unrelated to the changes that occurred in the skeletal muscle intracellular free amino acid pool ($r = 0.11$, not significant). Hindquarter nitrogen flux could be predicted and most of the variability in the data could be accounted for when both BCAA 6-hour flux and the change in the free amino acid nitrogen pool were utilized. The relationship was:

$$y = -9.58 + 0.27x_1 + 3.02x_2$$

where, y = amino acid nitrogen flux at 6 hours μmol/min/kg

x_1 = BCAA flux at 6 hours (μmol/min/kg)

x_2 = change in skeletal muscle intracellular free amino acid nitrogen (postop-preop, mmol/L/24 hours)

$n = 22$, $p<.05$, $r = 0.86$

Discussion

A standardized laparotomy in anesthetized dogs has been shown to initiate many of the catabolic responses observed in critically ill humans. Total body protein catabolism, as measured by urinary nitrogen excretion, is increased. The control animals receiving saline excreted approximately

III. (Continued)

12-15 grams of nitrogen in the first 24 hours following operation. Prior studies in this model have demonstrated that nitrogen balance remains negative for three days following the operative procedure in spite of food intake.[19] In contrast, pair-fed sham-operated animals achieve nitrogen equilibrium in the first postoperative day. Consistent with and contributing to the increased urinary nitrogen loss, hindquarter release of total amino acid nitrogen at 6 hours following operation was 6 to 8 times that observed in control animals after an overnight fast. [18] Other changes in the saline-treated dogs, such as a decrease in blood and skeletal muscle amino acid concentrations are similar to alterations reported during catabolic states in humans.[4] Thus, the canine model exhibits postoperative responses that are similar to alterations in critically ill humans and, therefore, is suitable for examining the effects of exogenous amino acids on nitrogen metabolism and skeletal muscle amino acid exchange.

In the saline-treated animals, hindquarter release of amino acid nitrogen was markedly increased 6 hours postoperatively. This was associated with a net skeletal muscle release of all the BCAA's. At the same time, whole blood BCAA concentrations were unchanged, indicating that consumption of BCAA in visceral organs was roughly equivalent to the accelerated rate of skeletal muscle release. In the animals receiving amino acids, hindquarter total amino acid release was attenuated, whole blood and skeletal muscle nitrogen pools were maintained, and the hindquarter was converted from an organ of BCAA release to one of uptake. BCAA and, more specifically, leucine uptake was not associated with increased skeletal muscle release of glutamine. These results differ from previously published findings in which oral leucine[20] or intravenous infusion of BCAA's[21] was associated with increased forearm glutamine production in normal humans. This disparity in results may be due to alterations in BCAA metabolism in skeletal muscle caused by the catabolic stimulus in the postoperative dogs.

Hindquarter BCAA uptake correlated with the rate of BCAA infusion and with whole blood BCAA levels. There was also a significant correlation between hindquarter BCAA uptaken and diminished total amino acid nitrogen release. In spite of these relationships, however, skeletal muscle nitrogen release was not related to the rate of BCAA infusion. Thus, while skeletal muscle amino acid nitrogen release was related to skeletal muscle BCAA uptake, this response did not occur in a predictable manner when BCAA's were administered intravenously. It is possible that hormones or other substrates besides the BCAA's may determine muscle uptake of BCAA's. This, in turn, could account for the wide range of results observed in the clinical trials reported to date.

In addition to BCAA uptake, hindquarter nitrogen balance was significantly correlated with changes in either the labile pool of skeletal muscle amino acid nitrogen or the muscle-free glutamine concentration. As the intracellular concentration of glutamine and total amino acids decreased, the net efflux of skeletal muscle amino acids increased. In all three

III. (Continued)

amino acid infusion groups, the fall in intracellular amino acid nitrogen observed in saline-control animals was prevented. This may have resulted from several different mechanisms, including increased synthesis of glutamine from BCAA's and a diminished intracellular/extracellular gradient for glutamine, as seen with the provision of glutamine in the amino acid solutions. Whatever the mechanism, these data suggest that skeletal muscle nitrogen balance is related either directly or indirectly to the intracellular amino acid concentration. In a cultured skeletal muscle cell line, glutamine has been shown to inhibit protein degradation.[22] It is possible that our findings in postoperative dogs result from a similar regulatory effect of glutamine.

This study demonstrates that skeletal muscle amino acid release and, hence, the net turnover of muscle protein, can be predicted from two independent measurements. These are the rate of BCAA flux across the skeletal muscle vascular bed and the concentration of nitrogen in the skeletal muscle free amino acid pool. Skeletal muscle BCAA uptake appears not to be determined simply by the rate of intravenous infusion of amino acids. In contrast, the skeletal muscle intracellular pool of amino acids can be predictably maintained by administration of standard amino acid solutions. Further work is necessary to investigate the mechanisms that influence BCAA uptake by skeletal muscle and the concentrations of amino acids and/or glutamine during critical illness. With better understanding of these metabolic responses, it may be possible to consistently and effectively reduce net skeletal muscle protein catabolism in critically ill patients.

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TABLE I: COMPOSITION OF INFUSED SOLUTIONS (EXPRESSED AS GRAMS INFUSED/24 HOURS/KG

		ESSENTIAL AMINO ACIDS		NON-ESSENTIAL AMINO ACIDS		
SOLUTION		BCAA*	OTHER	GLUTAMINE	OTHER	TOTAL NITROGEN
SALINE	5	0	0	0	0	0
11% BCAA* + GLUTAMINE	6	0.46	0.54	1.64	1.04	0.62
11% BCAA* + NEAA**	3	0.46	0.54	0	2.77	0.62
22% BCAA* = SAA***	6	0.92	1.09	0	2.07	0.62
44% BCAA* + GLUTAMINE	4	1.84	0.54	0.82	1.04	0.62
44% BCAA* + NEAA**	3	1.84	0.54	0	1.90	0.62

* BCAA = Branched chain amino acids (valine, leucine, isoleucine)

** NEAA = Non-essential amino acids found in FreAmine III^{R} (American McGaw)
(alanine, arginine, glycine, histidine, proline, serine)

*** SAA = Standard amino acids supplied as FreAmine III^{R}

TABLE II: VOLUME AND COMPOSITION OF 24-HOUR URINARY EXCRETION

INFUSION	N	VOLUME (ml/kg)	NITROGEN INTAKE (g/kg)	TOTAL NITROGEN EXCRETION (g/kg)	NITROGEN BALANCE (g/kg)	UREA N EXCRETION (g/kg)	CREATININE EXCRETION (g/kg)	AMMONIUM EXCRETION (g/kg)
SALINE	5	43.8±10.2	0*	0.492±.02*	-0.492±.02*	0.409±.03*	0.039±.002	0.037±.005
11% BCAA	9	57.5±6.9	0.627±.005	0.786±.02	-0.160±.02	0.697±.01	0.033±.001	0.049±.004
22% BCAA	6	64.2±5.1	0.632±.001	0.685±.03**	-0.053±.03**	0.603±.03**	0.034±.002	0.045±.005
44% BCAA	7	74.0±8.2	0.627±.003	0.825±.05	-0.200±.05	0.701±.04	0.037±.002	0.041±.003

* Saline diff. from treatment groups, $p < 0.05$.

** Diff. from other 2 treatment groups, $p < 0.05$.

TABLE III: WHOLE BLOOD AMINO ACID PROFILE (μMOL/L, MEAN±SEM)

	SALINE			11% BCAA						22% BCAA			44% BCAA					
				GLN			NEAA						GLN			NEAA		
	PRE	6°	24°	PRE	6°	24°	PRE	6°	24°	PRE	6°	24°	PRE	6°	24°	PRE	6°	24°
GLN	724.0 ±55	565.0 ±53	764.0 ±64	764.0 ±63	754 ±57	1108 ±110	808 ±111	626.0 ±20.6	679.9 ±55.2	693.0 ±77	493.6 ±29.7	648.7 ±38.3	961.0 ±130	723.6 ±44.2	1040 ±66	861.5 ±40.5	607.0 ±23/4	558.0 ±33/5
ALA	431.9 ±42.6	257.9 ±13.4	325.0 ±56	429.0 ±38.8	270.7 ±35.1	290.9 ±14.8	344.1 ±81	364.0 ±101	481.0 ±152	379.0 ±62	327.7 ±38.0	366.0 ±43	407.0 ±55	242.2 ±29.6	240.3 ±9.7	372.0 ±103	362.6 ±49.1	359.1 ±34.9
GLY	414.0 ±90	212.0 ±14.5	239.9 ±16.1	439.0 ±57	419.0 ±63	469.0 ±60	194.3 ±38.7	509.5 ±34.4	595.0 ±89	381.0 ±67	521.9 ±33.3	559 ±51	247.0 ±32.9	330.1 ±88	255.4 ±18.2	243.3 ±51.4	376.7 ±28.2	420.7 ±16
ARG	241.2 27	152.5 ±7.9	175.0 ±9.1	262.1 ±39.3	221.5 ±18.2	265.9 ±23.1	177.1 ±29.6	229.8 ±33.1	296.2 ±41.2	208.9 ±19.7	254.8 ±8.3	323.8 ±19.3	185.2 ±17.4	184.9 ±8.8	181.5 ±5.6	191.2 ±1.8	229.2 ±15.0	261.9 ±9.3
SER	131.4 ±9.3	96.8 ±8.1	140.4 ±8.4	166.7 ±22.9	142.3 ±5.7	179.3 ±11.7	118.0 ±24.8	222.5 ±22.2	293.6 ±36.9	126.2 ±7.9	162.4 ±11.0	223.9 ±12.1	130.6 ±6.3	135.5 ±7.9	143.2 ±11.9	153.6 ±14.1	188.2 ±8.9	226.6 ±18
ASP	29.8 ±5.4	27.6 ±4.6	29.3 ±4.2	30.3 ±3.7	25.9 ±2.8	30.6 ±3.4	27.1 ±3.8	23.4 ±4.1	22.3 ±3.9	21.6 ±1.9	18.9 ±1.8	24.6 ±1.3	39.1 ±12.2	27.6 ±2.1	21.7 ±1.0	27.4 ±4.1	24.6 ±2.8	21.7 ±1.7
ASN	39.7 ±4.9	26.7 ±2.1	54.7 ±2.6	48.0 ±3.8	31.6 ±1.1	50.4 ±3.9	54.1 ±8.7	33.1 ±4.6	47.6 ±8.0	37.9 ±6.8	22.9 ±2.1	37.1 ±4.1	56.1 ±4.7	32.0 ±1.9	47.6 ±3.3	55.6 ±2.8	26.3 ±1.0	38.4 ±4.6
GLU	65.8 ±4.7	68.0 ±4.1	72.3 ±3.9	66.1 ±4.3	67.1 ±5.0	66.9 ±2.6	66.0 ±10.7	75.8 ±11.4	63.6 ±4.9	51.2 ±4.3	51.74 ±3.2	55.7 ±2.5	62.0 ±2.8	68.3 ±3.9	59.5 ±4.0	72.6 ±9.6	66.4 ±8.8	50.8 ±7.4
HIS	109.8 ±8.3	108.0 ±11.9	122.0 ±10.1	105.1 ±14.3	115.9 ±10.e	133.9 ±10.e	88.2 ±6.0	116.8 ±6.6	133.1 ±11.9	100.3 ±7.1	121.2 ±15.6	136.7 ±11.7	104.2 ±5.8	119.9 ±11.2	103.1 ±1.6	92.0 ±1.6	113.9 ±2.5	109.3 ±4.3
TYR	72.8 ±7.3	54.9 ±1.2	66.2 ±4.8	91.3 ±24.6	53.3 ±5.0	63.9 ±4.9	52.1 ±5.7	45.1 ±0.1	54.3 ±3.9	65.7 ±5.8	54.2 ±2.6	69.2 ±2.6	63.3 ±6.4	52.8 ±5.2	45.9 ±4.6	62.5 ±6.6	45.1 ±5.4	48.5 ±4.3
TAU	264.3 ±39.3	411.0 ±46	441.3 ±29.5	346.0 ±73	467.0 ±58	474 ±62	317.5 ±45.1	401.3 ±45.9	459 ±65	248.9 ±32.7	327.7 ±38	430 ±49	317.8 ±17.4	360.4 ±48.7	295.5 ±22.6	343.3 ±31.6	444.9 ±11.6	412.5 ±32
MET	12.3 ±0.9	16.6 ±1.9	40.8 ±2.6	42.2 ±10.2	33.9 ±6.2	54.3 ±9.5	25.7 ±3.0	20.7 ±8.4	33.1 ±6.8	23.5 ±6.7	52.0 ±14	95.4 ±9.1	33.7 ±6.6	14.2 ±3.7	32.4 ±4.8	35.0 ±13.2	22.8 ±6.5	43.3 ±3.7
THR	239.0 ±29	160.5 ±28.4	281.7 ±28.7	264.0 ±66	204.6 ±30.5	306.3 ±35.9	241.9 ±24.5	245.2 ±15.9	277.0 ±67	253.5 ±29.4	239.7 ±15.6	347.1 ±31.3	227.7 ±38.8	254.9 ±34.4	224.4 ±10.7	208.4 ±8.2	185.7 ±9.9	204.1 ±18
PHE	44.7 ±4.1	52.9 ±4.6	69.3 ±4.7	45.9 ±5.4	74.2 ±5.8	89.7 ±5.7	41.9 ±3.1	66.3 ±2.0	82.5 ±4.4	45.5 ±3.4	94.6 ±2.1	103.1 ±4.5	43.1 ±2.5	63.5 ±7.3	73.8 ±4.9	37.2 ±1.3	58.0 ±3.9	72.9 ±6.7
VAL	149.5 ±11.3	143.4 ±8.4	210.3 ±13.9	165.9 ±24.5	225.1 ±32.0	250.5 ±22.9	135.2 ±16.8	203.9 ±18.0	271.0 ±10.2	137.6 ±17.3	304.1 ±26.4	391.5 ±21.3	128.2 ±8.2	446.7 ±42.6	636.8 ±24.4	155.1 ±11.3	513.1 ±16.7	805.1 ±16.
ILE	53.1 ±5.0	54.4 ±3.8	85.7 ±4.2	55.9 ±5.8	97.3 ±14.1	112.6 ±9.5	45.4 ±7.1	90.0 ±9.5	121.6 ±4.4	49.6 ±6.7	137.2 ±16.8	175.6 ±8.9	43.2 ±4.2	177.0 ±15.4	257.2 ±8.7	57.1 ±3.3	211.7 ±8.6	325.9 ±10.
LEU	102.7 ±7.4	96.2 ±7.1	153.2 ±7.3	105.2 ±5.6	153.2 ±29.7	166.6 ±15.8	99.0 ±19.0	140.7 ±16.3	179.6 ±5.8	100.5 ±15.4	196.2 ±24.6	263.4 ±16.7	95.1 ±7.1	280.6 ±21.6	420.4 ±15.9	121.5 ±1.6	341.2 ±13.7	524.8 ±19.
TOTAL BCAA	305.3 ±23	294.0 ±17.6	449.3 ±24.8	322.31 ±30.9	475.6 ±68.1	534.7 ±51.0	279.6 ±35.0	434.6 ±35.7	562.1 ±9.2	299.1 ±38.4	637.0 ±67	830.0 ±45	266.4 ±15.8	904.4 ±68.8	1314.4 ±42.3	333.7 ±12.2	1066.0 ±31.2	1638.5 ±49.6
TOTAL NITROGEN	4.72 ±.36	3.82 ±.08	4.87 ±.20	5.27 ±.38	5.06 ±.20	6.69 ±.44	4.40 ±.50	5.00 ±.31	5.96 ±.64	4.71 ±.31	5.08 ±.17	6.31 ±.19	4.93 ±.28	5.07 ±.21	5.92 ±.18	4.76 ±.26	5.37 ±.10	6.03 ±.01

TABLE IV: SKELETAL MUSCLE AMINO ACID PROFILE (MMOL/L, MEAN ± SEM)

	SALINE		11% BCAA				22% BCAA		44%BCAA			
			GLN		NEAA				GLN		NEAA	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
GLN	21.48 ±3.21	15.86 ±3.80	19.85 ±3.17	21.78 ±2.01	30.25 ±1.63	21.04 ±1.92	18.69 ±3.74	18.15 ±3.76	24.83 ±2.72	26.20 ±3.86	22.55 ±3.57	21.6 ±2.2
ALA	5.35 ±.55	5.58 ±.88	4.81 ±.44	5.62 ±.39	5.59 ±.23	8.11 ±2.35	4.71 ±.46	4.69 ±.79	5.16 ±.95	7.55 ±.89	4.45 ±.27	4.01 ±1.6
GLY	2.85 ±.31	2.28 ±.51	4.30 ±.65	3.22 ±.42	3.67 ±.37	3.63 ±.25	4.06 ±.74	4.52 ±1.15	3.70 ±.31	2.87 ±.17	4.08 ±.63	2.33 ±.82
ARG	1.17 ±.24	0.73 ±.08	0.91 ±.21	0.78 ±.03	1.45 ±.41	2.06 ±.51	1.21 ±.14	1.14 ±.15	1.06 ±.20	0.59 ±.13	1.10 ±.39	0.95 ±.35
SER	1.42 ±.16	1.10 ±.19	1.42 ±.26	1.65 ±.13	1.52 ±.09	2.33 ±.22	2.10 ±.60	1.38 ±.22	1.69 ±.12	1.63 ±.21	1.72 ±.32	1.34 ±.57
ASP	0.93 ±.20	1.04 ±.13	0.50 ±.19	0.80 ±.21	1.63 ±.16	1.95 ±.41	0.85 ±.52	0.62 ±.15	1.15 ±.12	2.86 ±.34	1.29 ±.31	1.69 ±.76
ASN	0.43 ±.05	0.50 ±.07	0.51 ±.07	0.67 ±.08	0.53 ±.04	0.43 ±.06	0.43 ±.09	0.45 ±.08	0.86 ±.15	0.60 ±.18	0.42 ±.09	0.36 ±.09
GLU	6.30 ±.86	3.91 ±.30	4.48 ±.98	4.38 ±.84	11.23 ±.75	9.08 ±1.25	5.96 ±.83	4.97 ±1.30	10.29 ±.83	9.29 ±1.03	9.00 ±2.08	8.98 ±1.9
HIS	0.81 ±.28	0.39 ±.02	0.45 ±.12	0.59 ±.11	0.69 ±.01	0.96 ±.14	0.79 ±.18	0.50 ±.03	0.79 ±.14	0.62 ±.08	0.71 ±.18	0.46 ±.23
TYR	0.17 ±.02	0.13 ±.02	0.27 ±.09	0.24 ±.05	0.36 ±.20	0.36 ±.09	0.32 ±.09	0.27 ±.05	0.33 ±.11	0.22 ±.10	0.29 ±.11	0.13 ±.06
MET	0.06 ±.03	0.04 ±.02	0.30 ±.02	0.06 ±.04	0.07 ±.01	0.13 ±.01	0.04 ±.03	0.06 ±.04	0.08 ±.01	0.12 ±.01	0.06 ±.01	0.09 ±.01
THR	1.20 ±.23	1.11 ±.17	1.36 ±.30	1.62 ±.18	2.24 ±.27	2.25 ±.21	1.59 ±.23	1.70 ±.26	2.57 ±.48	2.29 ±.42	1.98 ±.30	1.46 ±.09
PHE	0.11 ±.02	0.14 ±.05	0.11 ±.03	0.17 ±.02	0.12 ±.03	0.16 ±.01	0.09 ±.02	0.14 ±.02	0.13 ±.02	0.16 ±.01	0.10 ±.01	0.13 ±.01
VAL	0.19 ±.01	0.21 ±.04	0.20 ±.01	0.37 ±.05	0.20 ±.04	0.32 ±.04	0.22 ±.03	0.37 ±.07	0.21 ±.03	0.80 ±.04	0.22 ±.01	1.03 ±.03
ILE	0.09 ±.003	0.11 ±.02	0.09 ±.01	0.17 ±.03	0.11 ±.03	0.17 ±.02	0.10 ±.02	0.17 ±.03	0.11 ±.02	0.27 ±.03	0.10 ±.003	0.23 ±.03
LEU	0.16 ±.01	0.28 ±.12	0.16 ±.01	0.23 ±.05	0.18 ±.05	0.21 ±.03	0.16 ±.02	0.26 ±.04	0.17 ±.02	0.42 ±.05	0.17 ±.001	0.46 ±.03
TOTAL BCAA	0.44 ±.02	0.59 ±.17	0.42 ±.02	0.77 ±.13	0.50 ±.12	0.69 ±.16	0.47 ±.07	0.80 ±.14	0.49 ±.07	1.49 ±.11	0.48 ±.01	1.73 ±.09
TOTAL NITROGEN	69.8 ±8.5	52.8 ±8.4	63.5 ±7.0	68.3 ±4.4	96.4 ±5.2	82.8 ±5.9	65.2 ±10.3	62.5 ±9.6	83.6 ±7.0	85.9 ±8.9	75.9 ±11.8	70.4 ±8.4

TABLE V: HINDQUARTER AMINO ACID FLUX (μMOL/MIN/KG, MEAN±SEM)

	SALINE		11% BCAA				22% BCAA		44% BCAA			
			GLN		NEAA				GLN		NEAA	
	6°	24°	6°	24°	6°	24°	6°	24°	6°	24°	6°	24°
GLN	-2.69 ±1.07	-1.71 ±.70	-1.19 ±.46	-0.16 ±.82	-2.10 ±.62	-1.92 ±1.72	-1.92 ±.60	-1.24 ±.44	-0.73 ±.60	+0.74 ±1.76	-2.00 ±.32	-4.13 ±1.45
ALA	-2.19 ±.52	-0.72 ±1.26	-0.92 ±.23	-0.73 ±.44	-1.08 ±.28	-0.95 ±.25	-0.98 ±.84	-2.55 ±.84	-0.97 ±.22	-1.99 ±.98	-1.17 ±.09	-1.49 ±.67
GLY	-1.38 ±.36	-0.56 ±1.05	-0.66 ±.20	-0.28 ±.31	-0.86 ±.19	+0.47 ±.72	-0.05 ±.40	-0.89 ±.53	-0.44 ±.14	-0.78 ±.39	-0.39 ±.21	-0.25 ±.25
ARG	-0.83 ±.14	+0.12 ±.72	-0.29 ±.13	-0.09 ±.25	-0.13 ±.07	+0.28 ±.18	-0.28 ±.38	-0.26 ±.22	-0.11 ±.24	-0.28 ±.24	-0.13 ±0.06	+0.02 ±.16
SER	-0.49 ±.11	+0.09 ±.49	-0.11 ±.28	+0.11 ±.23	-0.14 ±.07	+0.37 ±. 29	+0.50 ±.45	+0.65 ±.50	-0.14 ±.10	-0.13 ±.22	-0.22 ±.11	+0.16 ±.06
ASP	+0.04 ±.04	+0.19 ±.12	-0.01 ±.01	+0.02 ±.05	-0.00 ±.01	-0.03 ±.11	+0.02 ±.04	+0.00 ±.08	+0.03 ±.03	-0.24 ±.21	+0.01 ±.03	-0.09 ±.11
ASN	-0.22 ±0.9	-0.14 ±.11	-0.07 ±.04	-0.14 ±.04	-0.11 ±.04	-0.38 ±.29	-0.15 ±.09	-0.12 ±.13	-0.07 ±.05	-0.32 ±.13	-0.08 ±.05	-0.12 ±.05
GLU	+0.10 ±.18	+0.21 ±.11	+0.06 ±.07	+0.08 ±.02	+0.06 ±.07	+0.17 ±.09	+0.11 ±.06	+0.23 ±.13	+0.16 ±.04	+0.21 ±.15	-0.01 ±.03	-0.10 ±.09
HIS	-.44 ±.09	-0.19 ±.44	-0.09 ±.17	+0.08 ±.20	-0.03 ±.14	-0.05 ±.19	-0.34 ±.27	-0.24 ±.36	-0.03 ±.10	-0.25 ±.22	-0.06 ±.12	-0.01 ±.01
TYR	-0.26 ±.09	-0.08 ±.40	-0.13 ±.02	-0.11 ±.04	-0.13 ±.04	-0.04 ±.08	-0.19 ±.06	-0.14 ±.05	-0.10 ±.01	-0.18 ±.09	-0.07 ±.03	-0.0 ±.0
TAU	-1.05 ±.33	+0.78 ±2.18	-0.23 ±.28	+0.18 ±.51	-0.27 ±.10	+1.39 ±.74	-0.08 ±.61	+0.24 ±.39	-0.08 ±.15	-0.48 ±.34	+0.21 ±.07	-0.5 ±.1
MET	-0.32 ±.09	-0.33 ±.33	-0.23 ±.11	-0.59 ±.28	-0.61 ±.40	-2.13 ±1.08	-0.74 ±.41	-1.66 ±.86	-0.21 ±.06	-1.69 ±.35	-0.26 ±.16	-0.5 ±.21
THR	-1.06 ±.10	+0.72 ±.86	-0.30 ±.13	-0.27 ±.32	-0.07 ±.43	+1.28 ±.67	+0.19 ±.37	-0.13 ±.46	-0.06 ±.11	+0.57 ±.35	+0.28 ±.17	+0.2 ±.1
PHE	-0.37 ±0.5	-0.20 ±.36	-0.12 ±.04	-0.19 ±.08	-0.13 ±.03	-0.12 ±.03	-0.14 ±.09	-0.13 ±.15	-0.14 ±.08	-0.27 ±.15	-0.07 ±.03	-0.1 ±.12
VAL	-0.46 ±.17	+0.33 ±.68	+0.14 ±.08	+0.02 ±.11	+0.31 ±.26	+0.49 ±.23	+0.62 ±.46	+1.12 ±1.01	+0.67 ±.23	+0.37 ±.45	+1.34 ±.17	+1.2 ±.65
ILE	-0.24 ±.03	+0.12 ±.30	+0.08 ±.02	+0.06 ±.08	+0.27 ±.19	+0.42 ±.15	+0.49 ±.21	+0.39 ±.15	+0.42 ±.04	+0.51 ±.21	+.80 ±.07	+0.9 ±.4
LEU	-0.43 ±.09	+0.04 ±.54	+0.06 ±.07	-0.08 ±.08	+0.30 ±.25	+0.50 ±.24	+0.51 ±.33	+0.57 ±.39	+0.61 ±.06	+0.69 ±.33	+1.14 ±.13	-1.4 ±.6
TOTAL BCAA	-1.14 ±.26	+0.49 ±1.51	+0.28 ±.14	-0.03 ±.19	+0.88 ±.57	+1.40 ±.50	+1.64 ±.86	+2.09 ±1.54	+1.71 ±.22	+1.57 ±.83	+3.28 ±.18	+3. ±1.
TOTAL NITROGEN	-19.05 ±4.06	-3.59 ±12.1	-6.52 ±1.81	-3.25 ±3.07	-7.39 ±.52	-1.80 ±6.40	-7.70 ±5.90	-8.42 ±1.90	-2.50 ±2.50	-7.40 ±8.70	-3.27 ±1.62	-7. ±2.

FIGURE LEGENDS

Figure 1: Total BCAA level in arterial blood increases with the rate of administration of branched chain amino acids. Data represent mean ± SEM. Data from animals receiving saline are not included.

Figure 2: Relationship between hindquarter BCAA flux and BCAA infusion rate.

Figure 3: BCAA flux across the hindquarter 6 hours postoperatively increases as the concentration of BCAA in arterial blood rise.

Figure 4: Hindquarter nitrogen flux is related to the hindquarter BCAA flux 6 hours after operation.

Figure 5: Relationship between hindquarter nitrogen flux 6 hours after operation and the change in muscle intracellular amino acid nitrogen measured 24 hours postoperation.

FIGURE 1

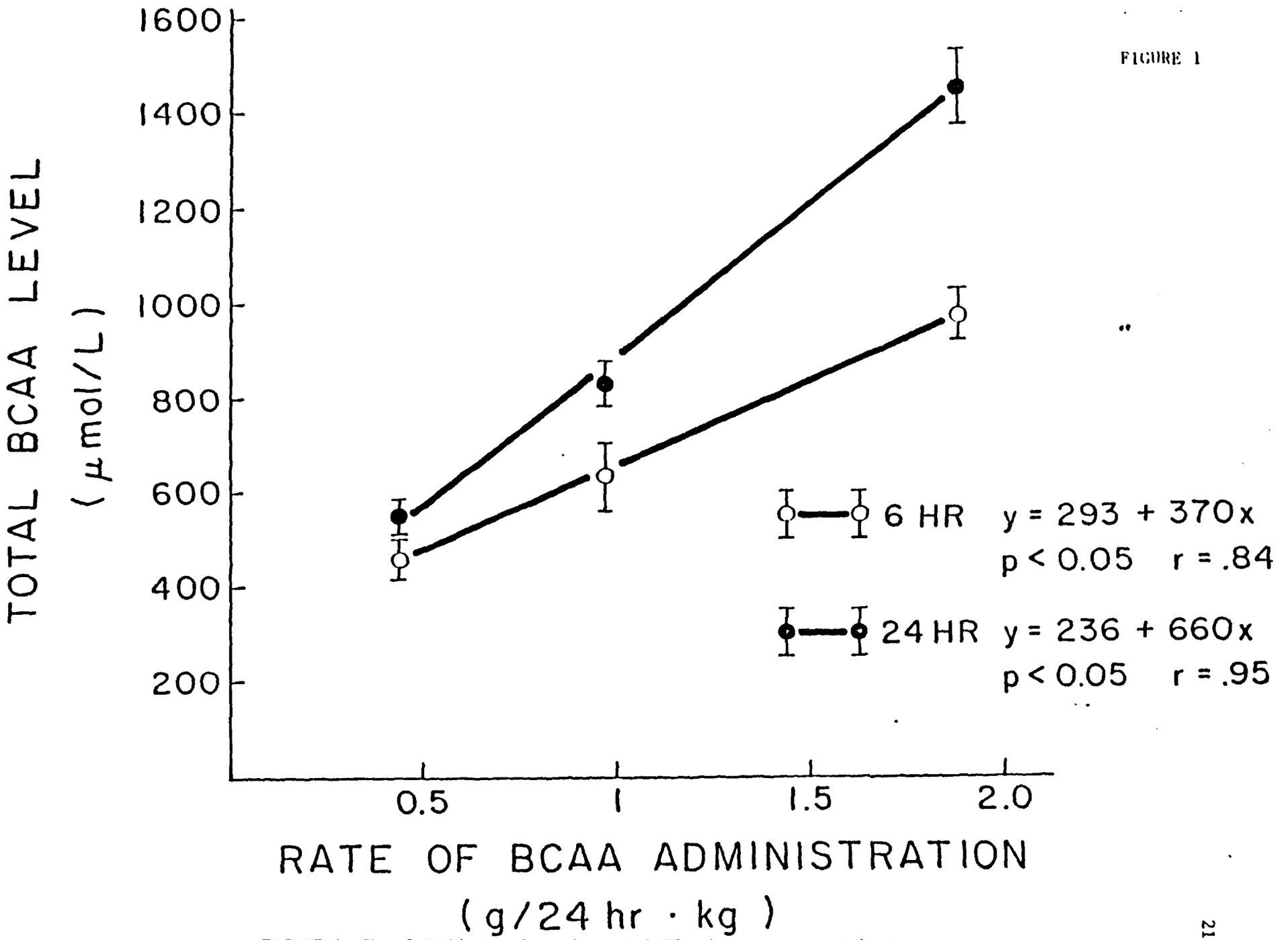

FIGURE 1 Total BCAA Level in Arterial Blood Increases with the Administration of Branched Chain Amino Acids

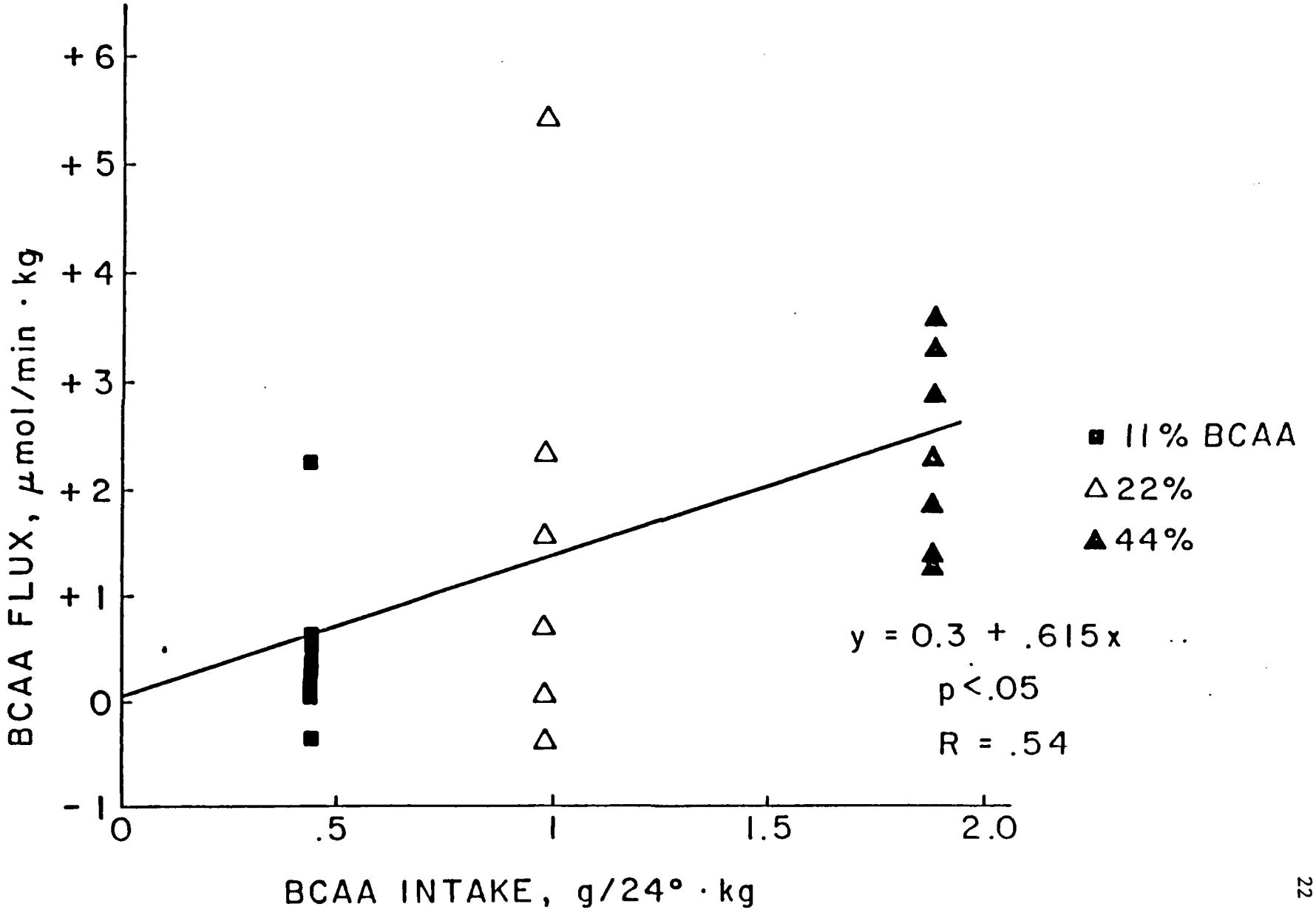

FIGURE 2 Relationship Between BCAA Flux and BCAA Infusion Rate

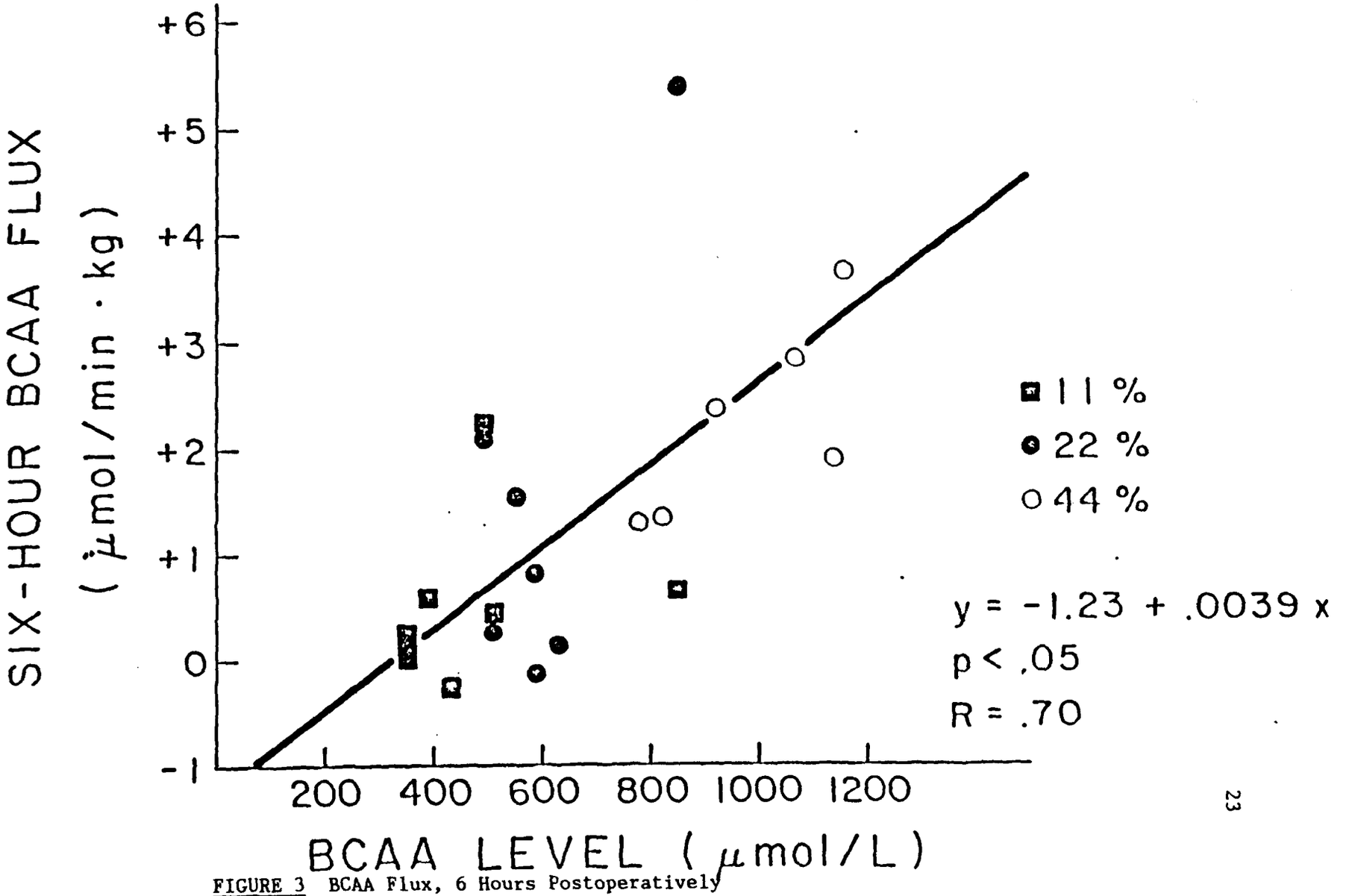

FIGURE 3 BCAA Flux, 6 Hours Postoperatively

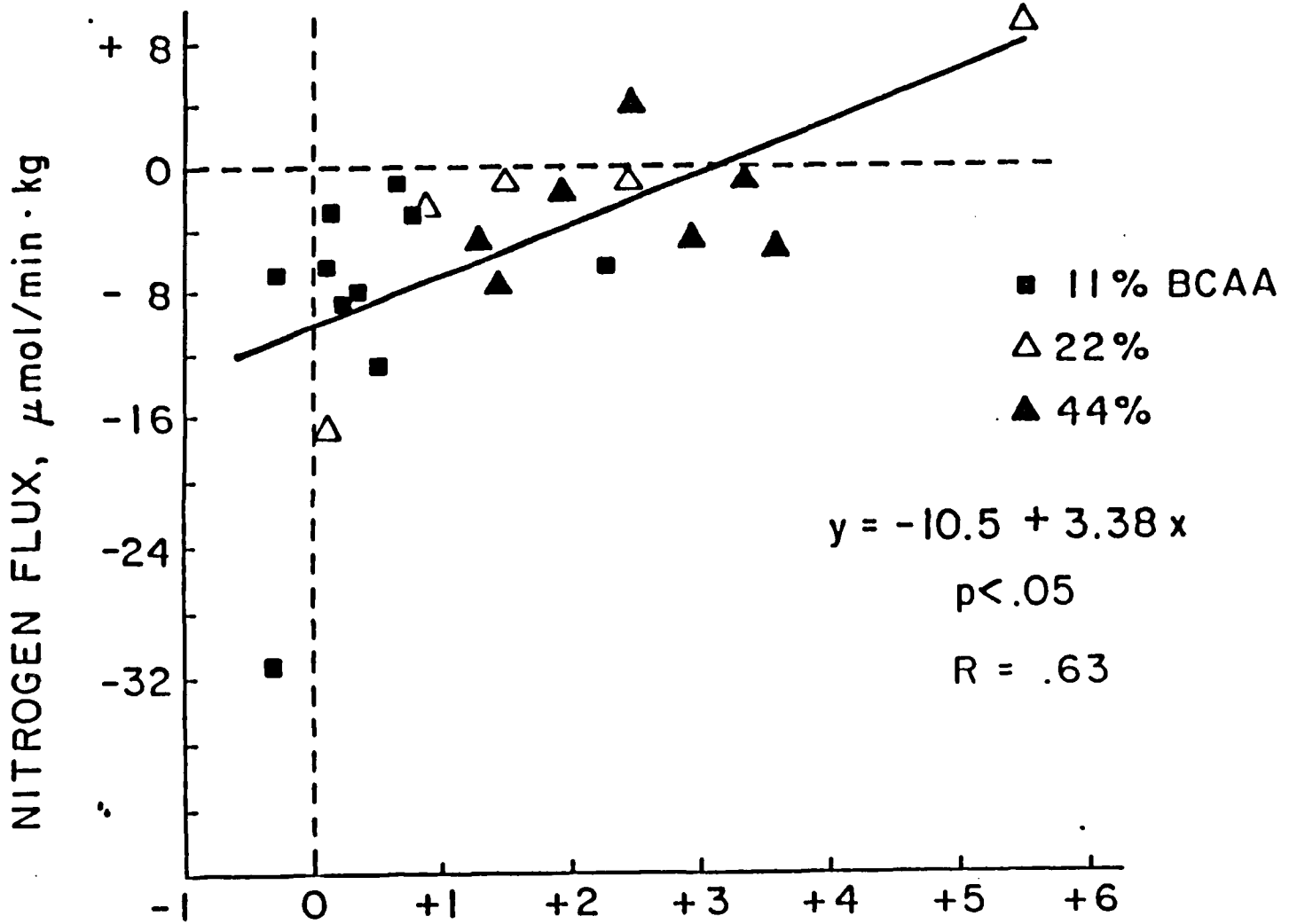

FIGURE 4 Relationship of Hindquarter Nitrogen Flux to Hindquarter BCAA Flux 6 Hours After Operation

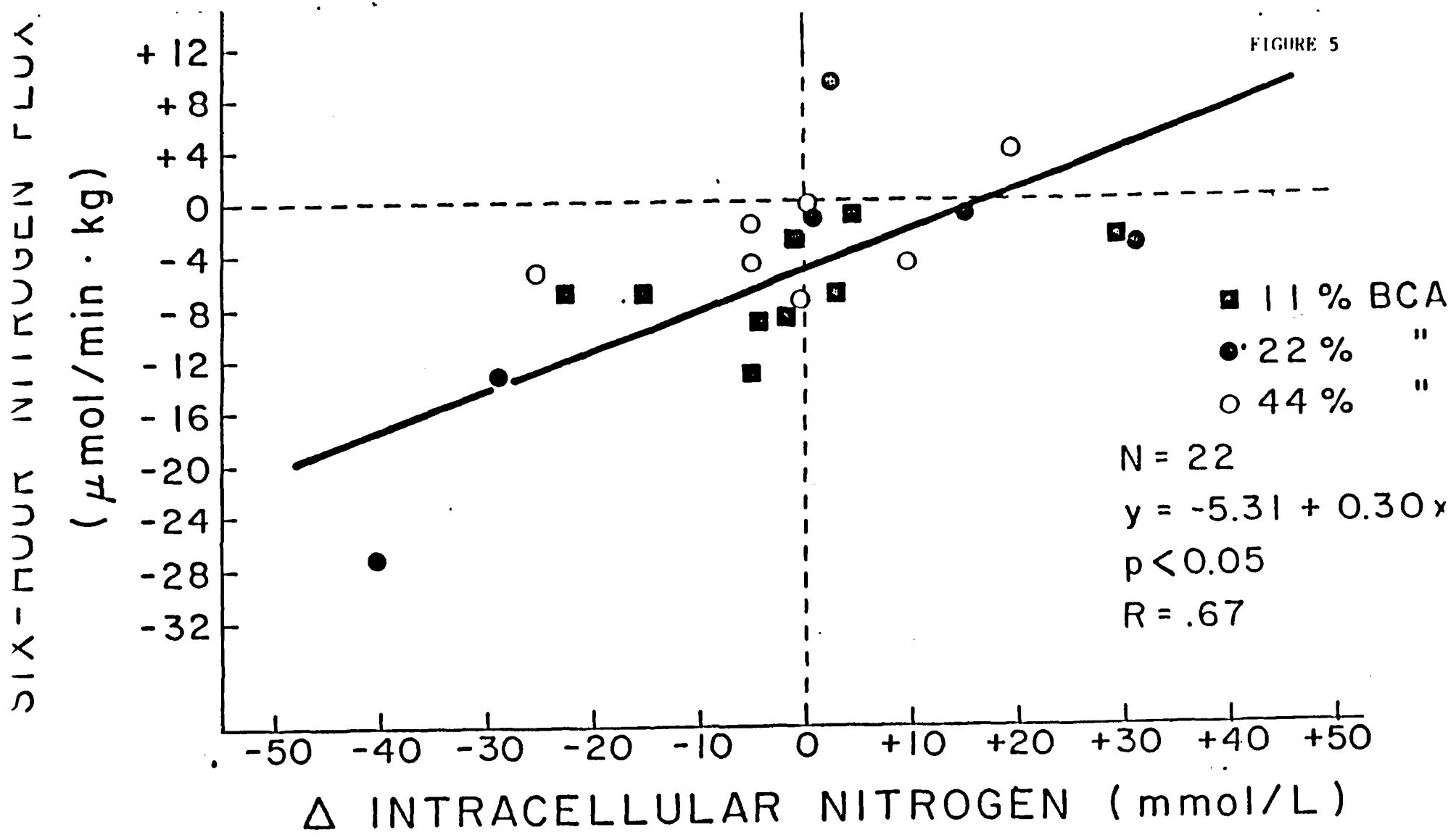

FIGURE 5 Relationship Between Hindquarter Nitrogen Flux and The Change in Muscle Intracellular Amino Acid Nitrogen Measured 24 Hours Postoperation.

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